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A new species of *Entocybe* (*Entolomataceae*, *Agaricomycetes*) from Québec, Canada

Timothy J. Baroni[1*] & Yves Lamoureux[2]

[1]*Department of Biological Sciences, State University of New York – College at Cortland PO Box 2000, Cortland, NY 13045 USA*

[2]*Cercle des mycologues de Montréal, Jardin botanique de Montréal, 4101 rue Sherbrooke Est, Montréal, Québec, Canada H1X 2B2*

* Correspondence to: *tim.baroni@cortland.edu*

Abstract — *Entocybe melleogrisea* sp. nov. is described from a subboreal forest type in Québec, Canada. The stature of this species is robust and tricholomatoid and thus is very different from the mostly collybioid species placed in the genus. The distinctive basidiospore morphology, basidiospore histochemistry, and prolific, obvious clamp connections on all hyphae of the basidiomata place this species in *Entocybe.* Preliminary data on ITS sequences support this placement.

Key words — *Agaricales*, mushrooms, North America, key to species

Introduction

A fair number of publications are available on systematics of the *Entolomataceae* Kotl. & Pouzar in North America (Atkinson 1902, 1909; Baroni 1981; Baroni & Petersen 1987; Hesler 1967; Largent 1977, 1994; numerous publications by Peck from 1872 to 1908 [see Murrill 1917 and Noordeloos 2008]; Mazzer 1976; Murrill 1917), but as yet there is no comprehensive monograph of this large and morphologically diverse group of agarics with attached lamellae and flesh-pink angular spores. Although the genus *Entoloma* (Fr.) P. Kumm. s.l. is much better known in Europe (Noordeloos 1992, 2004), even there new species are still being discovered (Noordeloos & Hausknecht 2000; Noordeloos & Morozova 2010). The *Entolomataceae* in North America is a seriously understudied mushroom family. With a minimum of at least 500 species already described from North America (mostly summarized in Hesler 1967 and Largent 1994), it is not surprising that newly discovered taxa are still being found. This report documents one of these distinctive new species.

Baroni et al. (2011) erected the genus *Entocybe* T.J. Baroni et al. in the *Entolomataceae* to recognize a group of species with several unique basidiospore features and a distinctive DNA signature. The basidiospores of *Entocybe* taxa are reminiscent of basidiospores in the genus *Rhodocybe*, i.e., they are subglobose (isodiametric), 6–12 angled in polar view, often obscurely angled in profile view, and the basidiospore surface is adorned with scattered pustules with broken or partial ridging. In addition, all taxa produce obvious clamp connections on the hyphae in all tissues. Taxa with this suite of characters are now confirmed to belong to a basal clade in the *Entolomataceae* using molecular DNA evidence (Baroni et al. 2011; Co-David et al. 2009). The morphological characters of this new species from North America clearly indicate it belongs in the genus *Entocybe*.

Materials & methods

Colors given are general color hues or matched to plates in Kornerup & Wanscher (1978). All microscopic structures were measured in mounts using 10% aqueous ammonia (household, non-sudsing, non-scented) or 3% KOH. Structures were also examined in mounts of Congo Red/ammonia, Melzer's Reagent and Cotton Blue/lactic acid to determine histochemical reactions of basidiospore and hyphal walls. For basidiospore measurements, the hilar appendix or apiculus was excluded. In those measurements Q refers to the length divided by the width of an individual spore. The notation n = 21 indicates that 21 individual spores were measured from a single sample. Means for length and width are given with their standard deviations, as is the mean of Q values, with the mean of the length divided by the width of all basidiospores measured being designated as Q^m.

Descriptive statistical analysis of basidiospores was determined using EXCEL 5.0. All light microscopic images were made with an Olympus BX 50 transmitted light microscope using DIC or bright field optics and captured with a Diagnostic Instruments, Inc. Insight Spot 3-shot color digital camera.

Scanning electron micrographs were captured on an ISI DS 130c scanning electron microscope run at 15 or 25 Kev. Tissue samples for SEM were prepared using a modified procedure originally described in Baroni (1981). Dried lamella fragments were soaked in 95% ETOH for 1 min, transferred to dH_2O for 1 min, then 3% KOH for 1 min, and finally washed three times in dH_2O for 1 min each. These rehydrated lamella fragments were then sandwiched between two filter papers in a modified Beem capsule and placed in 10% ETOH for a minimum of 2 minutes. The samples were dehydrated successively in 30, 50, 70, 80, 90, 95% ETOH solutions for 2 min each, and finally washed 2 more times in ice cold 100% ETOH before critical point drying in liquid CO_2 in a Tousimis Samdri PVT-3B critical point drier.

Results

The following new species is described from a single collection of multiple fruiting bodies found in the province of Québec, Canada. The large tricholomatoid stature and grayish yellow colors (Fig. 1) make the

Fig. 1. *Entocybe melleogrisea* (holotype). Basidiomata. Scale bar = 10 mm

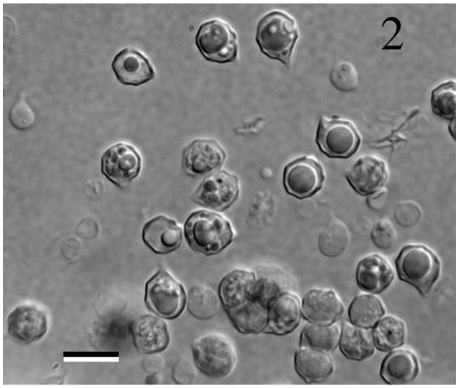
taxon distinctive in the field and unlike any other known species in North America. The subglobose or isodiametric basidiospores with obscure angles in profile and face views, the minute angles in polar view (Fig. 2), and the evenly cyanophilic walls of the basidiospores with the characteristic undulate-pustulate ornamentation (Fig. 3) coupled with obvious clamp connections in all tissues (Fig. 3) place this taxon in *Entocybe* (Baroni et al. 2011). The broken reticulum-like ornamentation (Fig. 4) as viewed under the scanning electron microscope is also a diagnostic feature for this group of temperate species of *Entolomataceae* (Baroni et al. 2011).

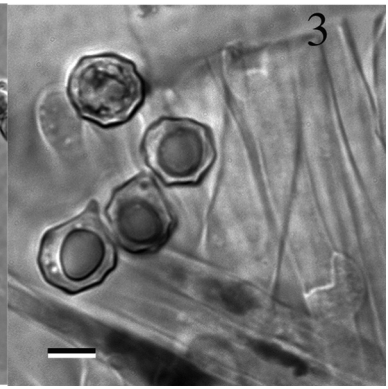
ITS (KC701386) and nLSU (KC701387; KC701388) sequences for our collection do not match any species in blast searches in GenBank. However the taxa that come closest in blast searches at 81–82% similarity are: *Entoloma nitidum* (AF335449.1 and AY228340.1), *Rhodocybe trachyospora* (JF899553.1), and *Entoloma turbidum* (FJ824815.1). These taxa are all considered members of *Entocybe* (Baroni et al. 2011), thus supporting our placement of this new species in that genus.

Entocybe melleogrisea T.J. Baroni & Y. Lamoureux, **sp. nov.** Figs 1–5

MycoBank MB 800629

Differing from other *Entocybe* species by the tricholomatoid stature, the grayish cream, opaque, non-hygrophanous, felted or fibrillose-squamulose pileus surface, the cream then pale brown stipe with a white, subbulbous, radicating base.





Figs. 2–3. *Entocybe melleogrisea* (holotype). **2.** Basidiospores. Scale bar = 10 µm. **3.** Basidiospores and clamp connection. Scale bar = 5 µm

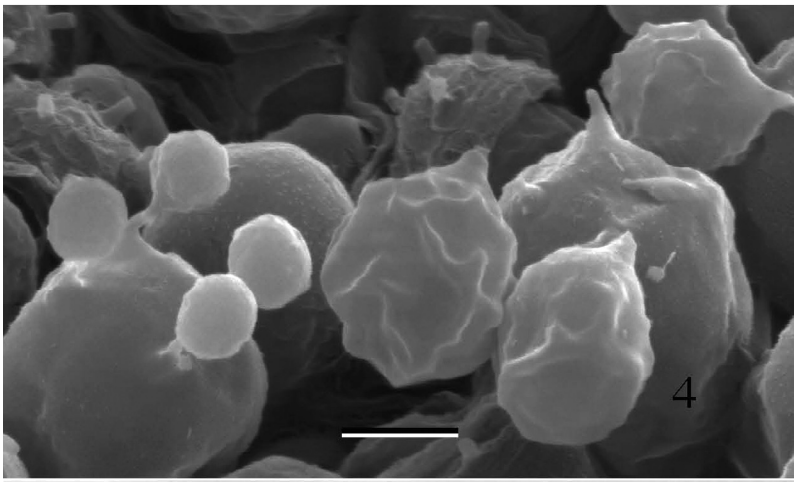
Type: Canada, Québec, City of Saint-Côme, scattered on soil and moss with dead leaves of *Betula papyrifera, Populus tremuloides,* and *Acer saccharum* or some fruiting directly from very rotten wood in a typical subboreal *Abies balsamea–Betula papyrifera* forest type of approximately 75 year old class, and also present in the area were *Picea* spp. and *Pinus strobus*, 18 August 2010, Yves Lamoureux 4150 (**Holotype**, CMMF; **isotype**, CORT). GenBank Accessions: KC701386 (ITS), KC701387 (nLSU, haplotype 1), KC701388 (nLSU haplotype 2).

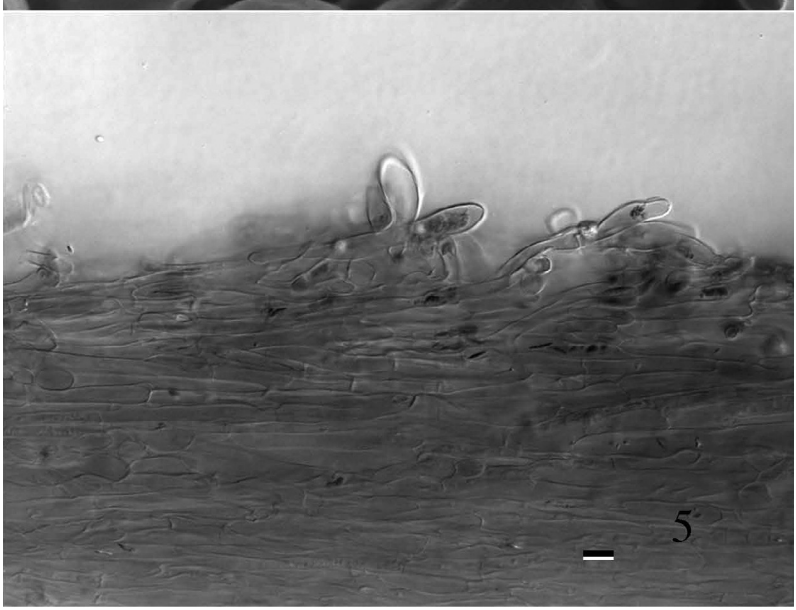
Etymology: from the distinctive grayish cream pileus and stipe colors dominating or in later development as ground color.

Basidiomata tricholomatoid on soil or humus, subboreal forest.

Pileus 40–50 mm, grayish yellow, grayish cream, blond or grayish beige (4B-C3-6), or medium brown to dark blonde (5D4) over grayish cream ground color, sometimes paler toward margin or other areas (irregularly pigmented), convex to campanulate, surface not consistently smooth but irregular on some, becoming plane with no or a very small rounded umbo, surface finely felted, then finely fibrillose-squamulose toward margin, not viscid, not hygrophanous, but a few watery spots sometimes present, not striate, but margin clearly with fine raised lines on one basidioma (similar to species of *Leucopaxillus* Boursier and some of the brown *Tricholoma* (Fr.) Staude species); Context white, very thin (1 mm), fragile; Lamellae whitish then pinkish, adnexed then sinuate, close, moderately thick, very broad (up to 5 mm), edge even; Stipe 35–60 × 4–6(–8 at base) mm, cream then pale brown but white over base, striate and with appressed fibrils, equal but with base slightly bulbous and obliquely radicating, solid becoming stuffed, then hollow; Odor and Taste not distinctive or absent. Spore print dull pink (pale brownish pink).

Basidiospores (6.4–)7.2–8(–8.8) × (5.6–)6.4–7.2(–8) µm (x = 7.5 ± 0.54 × 6.9 ± 0.53 µm, Q = 1–1.23, Q^m = 1.09 ± 0.07, n = 21) isodiametric or





Figs. 4–5. *Entocybe melleogrisea* (holotype). **4.** SEM of basidiospores (note broken ridges and isolated pustules). Scale bar = 5 µm. **5.** Pileipellis with pilocystidia and intracellular pigments. Scale bar = 10 µm

subisodiametric, obscurely angular in profile view with 6–9 facets, isodiametric and obscurely angular in face view with 5–7 facets, minutely angled in polar view with 6–9 facets, undulate-pustulate under the light microscope in all views, walls evenly cyanophilic on smaller, younger spores and larger mature spores

with breached wall structure, otherwise wall of the larger spores not absorbing the dye, ornamentation composed of broken ridges and isolated pustules under an SEM; Basidia (2–)4-sterigmate, long clavate, hyaline, filled with refractive oil bodies, 39.7–44 × 10.5–12 µm; Hymenial cystidia absent; Lamella trama a hyaline layer of parallel, cylindrical or inflated hyphae, 3–13.8 µm in diam; Pileipellis (Fig. 5) a brown layer of ± repent, cylindrical hyphae producing scattered mounds or clusters of ± ascendant, entangled cylindrical, subclavate or clavate pilocystidia, 6–16 µm in diam, brown pigments intracellular and often coagulated into discrete masses of various sizes; Pileus context hyaline, composed of radially arranged cylindrical and inflated hyphae, 6–24 µm in diam; Stipitipellis similar to pileipellis, a compact brown layer of repent, cylindrical hyphae, 2–6 µm in diam, producing scattered mounds or clusters of entangled, ascendant cylindrical, subclavate or clavate end cells similar to the pileipellis, brown pigments intracellular, frequently coagulated; Clamp connections present in all tissues, large and obvious.

Discussion: The distinctive basidiospore morphology and presence of obvious large clamp connections on hyphae of the basidiomata place this taxon in *Entocybe*. From the senior author's experience, large obvious clamp connections are not typical in the *Entoloma* s.l. clade and thus seem to be another diagnostic feature that can be used to recognize *Entocybe* species. The brownish pink isodiametric basidiospores that are obscurely angled in profile view but possess 6–9 minute angular facets in polar view and pustule-like ornamentation are diagnostic for *Entocybe* (Baroni et al. 2011). Also for *Entocybe* species the ultrastructure of the basidiospore ornamentation is consistent and composed of a bumpy pustulate ornamentation partially interconnected with an incomplete network of ridges connecting at least some pustules. This incomplete reticulum is best seen under a scanning electron microscope (Fig. 4). Finally, as noted in the Results section, ITS sequences also appear to support the placement of *E. melleogrisea* with other like taxa in *Entocybe* and confirm nothing like it occurs in GenBank.

Molecular analyses (Baroni et al. 2011, Co-David et al. 2009) show that *Rhodocybe* species in the *Rhodocybe*/*Clitopilus* clade that also have clamp connections present on the hyphae, e.g., *Rhodocybe nitellina* and *R. melleopallens,* produce very differently shaped and ornamented basidiospores. The basidiospores are ellipsoid or amygdaliform and with scattered non-connected pustules or rounded bumps. *Entocybe* (Baroni et al. 2011) belongs in the *Entoloma* clade, not the *Rhodocybe*/*Clitopilus* clade of the *Entolomataceae,* and these morphological features emphasized for the genus are consistent.

Entocybe melleogrisea is somewhat similar to *E. turbida* (Fr.) T.J. Baroni et al. and *E. pseudoturbida* (Romagn.) T.J. Baroni et al. because of the tricholomatoid habit. Both *E. turbida* and *E. pseudoturbida* differ from *E. melleogrisea* in

having brown or grayish brown colors on the pileus and stipe, lacking yellow or cream hues, and the pilei for both are hygrophanous and thus changing color with loss of moisture. *Entocybe turbida* has a translucent-striate moist and mostly glabrous pileus surface except for the disc, which can be subtomentose, while *E. pseudoturbida* may be translucent-striate at the margin, but the pileus is subviscid and opaque when moist. Both species also produce farinaceous odors and taste (or a rancid taste in the case of *E. turbida;* Noordeloos 1992). A few collections of *E. turbida,* or something very similar to that taxon, have been found in southern Québec and in the southeastern US; however *E. pseudoturbida* has not yet been reported for North America.

Due to the felted opaque pileus surface and tricholomatoid habit, *E. melleogrisea* superficially reminds one of a member of the genus *Trichopilus* (Romagn.) P.D. Orton. However, for *Trichopilus* species the pileus surface is distinctly tomentose and not felted, the basidiospores are ornamented with interconnected ridges, not broken ridges, and the pileipellis is a trichodermium, not a cutis. Members of *Trichopilus* frequently have hymenial cystidia, and these are often lecythiform on the lamella edge, whereas none of the taxa placed in *Entocybe* have been found to produce hymenial cystidia.

Key to *Entocybe* species

Of the 12 *Entocybe* taxa documented from the northern hemisphere, most occur in North America (Baroni et al. 2011). We are aware of at least two other undescribed taxa in North America that appear to belong in this genus, but at this time we have inadequate materials to produce scientific descriptions.

1a. Basdiomata with blue or bluish fuscous or violaceous or vinaceous colors on the pileus, lamellae and/or stipe 2

1b. Basidiomata with brown, gray, yellowish brown or grayish yellow or grayish cream colors on the pileus and often also the stipe, although the stipe may be white, but constantly lacking any blue, violaceous or vinaceous hue. 5

2a (1a). Pileus grayish blue, stipe blue or concolorous with pileus except for yellow hues in the base, lamellae white at first (Noordeloos 1992) . . . . . *E. nitida*

2b. Pileus grayish brown or dark purplish brown or reddish brown or fuscous purplish, lacking yellow hues in the base of the stipe, lamellae more highly colored 3

3a (2b). Pileus purplish brown to reddish brown at first, lamellae bluish gray at first, stipe dark blue to bluish gray, pileus flesh deep blue or bluish gray or purplish blue or violaceous gray. . . . . *E. trachyospora* var. *purpureoviolacea*

3b. Pileus grayish brown, lamellae pallid or grayish brown not with blue colors, pileus flesh not purplish or violaceous but may be concolorous with pileus surface 4

4a (3b). Stipe surface, lamellae and pileus context with vinaceous hues . . . . . *E. trachyospora* var. *vinacea*

4b. Vinaceous colors lacking in these tissues, pileus dark grayish brown but hygrophanous and becoming paler, lamellae pallid or grayish brown at first, context buff . . . . . *E. trachyospora* var. *griseoviolacea*

5a (1b). Lignicolous. 6
5b. On humus, mosses, leaf litter, but not lignicolous 7

6a (5a). Pileus tan or yellowish brown, stipe yellow or pale orange, end cells in pileipellis variously shaped as pilocystidia, hyphae of lamella trama and pileus context dextrinoid . . . . . *E. speciosa*
6b. Pileus "off white to pale buff" becoming a uniform tan, stipe off white, lacking pilocystidia, hyphae of lamella trama and pileus context not dextrinoid (Largent 1989) . . . . . *E. lignicola*

7a (5b). Stature tricholomatoid, medium to large with pileus diameters reaching 50–70 mm, lamellae adnexed or deeply emarginated to almost free or sinuate 8
7b. Stature collybioid, medium to small basidiomata, pileus diameters reaching 40 mm, lamellae attachment more variable, finely adnexed with decurrent tooth or emarginate or sinuate or adnate 10

8a (7a). Pileus grayish cream or grayish yellow or dark blond to medium brownish, but with grayish cream ground colors, dry, not hygrophanous, surface felted or minute fibrillose-squamulose, stipe concolor, odor and taste absent . . . . . *E. melleogrisea*
8b. Pileus gray-brown or sepia or reddish brown or dark umber brown, hygrophanous and becoming paler, stipe gray-brown or nearly concolorous with the pileus, odor farinaceous or absent, taste farinaceous or rancid or mild 9

9a (8b). Pileus moist, obscurely to distinctly translucent striate to mid pileus, grayish brown or sepia or reddish brown, pallescent after losing moisture, smooth, glabrous or subtomentose on disc when dry, stipe pale grayish brown and often with yellow hues over base especially when bruised, odor absent or somewhat farinaceous, taste mild or distinctly rancid (Noordeloos 1992) . . . . . *E. turbida*
9b. Pileus subviscid when moist, not translucent striate or only at very margin, dark umber brown or sepia but slightly pallescent after losing moisture, radially fibrillose or subrugulose on drying, stipe gray or grayish brown, odor and taste strongly farinaceous (Noordeloos, 1992) . . . . . *E. pseudoturbida*

10a (7b). Stipe grayish over apex, yellowish downwards, distinctly tapered, ± rooting and often flexuous. . . . . *E. vinacea*
(if stipe entirely grayish *E. vinacea* var. *fumosipes*, if stipe violaceous then *E. vinacea* var. *violeipes* – Noordeloos 1992)
10b. Stipe off white, orange gray or brownish orange or hazel or vinaceous buff, enlarged at base or equal and tapering upward, not rooting, not flexuous 11

11a (10b). Stipe off white, orange gray or brownish orange with age and handling, lubricous, pileus grayish brown or dark yellowish brown or dark brown, lubricous, caulocystidia often sphaeropedunculate or pedicellate-capitate . . . . . *E. trachyospora var. trachyospora*
11b. Stipe hazel or vinaceous buff becoming buff, dry, pileus umber or brown vinaceous on the disc, pale umber or hazel or grayish sepia elsewhere, dry, caulocystidia mostly clavate or cylindrical . . . . . *E. priscua*

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